II. *The effects of temperature in the pupal stage on the colouring of* Pieris napi, Vanessa atalanta, Chrysophanus phlœas, and Ephyra punctaria. By Frederic Merrifield, F.E.S.

[Read December 7th, 1892.]

Plate IV.

I wish to premise that nothing approaching to the amount of variation one would find in large collections can be expected as the result of these temperature experiments. In such collections we find the results of a combination of different causes, including individual tendencies to sport, and they contain selections from thousands of individuals of different broods and from different localities, the object of most collectors being to assemble the most abnormal specimens they can meet with. My object, on the contrary, has been to obtain sets of individuals naturally as much alike as possible, so as to make sure that any changes were the results of the different temperature conditions applied; and to secure this natural invariableness I always, where it was possible, obtained a set from the same parents. Notwithstanding all the consequent limitations in result, I think there are some varieties, plainly attributable to temperature, among the specimens I exhibit this evening, that would be noticed in any cabinet.

Let me further remark that when in the course of this paper I speak of certain of my artificial temperatures as corresponding with the temperature of certain seasons or countries, I am well aware that the correspondence is in some cases incomplete. Natural temperatures are so fluctuating that it is difficult to imitate them artificially, but I do not think the difference in this respect is for my purposes an important one, for in many instances I have used both artificially equable, and naturally fluctuating temperatures, and in these cases I have invariably found that a fluctuating temperature produced results similar to those obtained from an equable

temperature corresponding to the mean of the fluctuating one.

Again, in reference to the known English mean temperatures of the spring and summer months, it must be borne in mind that these are shade temperatures, and are below, and under certain circumstances of exposure or absence of cloud considerably below, those to which objects exposed to both sunshine and shade under natural conditions would be subjected.

In the experiments hereafter described, the pupæ were exposed to the different temperatures in nearly all cases within a day or two, and often within a few hours, after pupation. The pupal period in which the temperature has been found to be in general most effective as regards the colouring is that which intervenes between the central inactive stage and that in which the colouring of the imago begins to be perceived in the pupa.

P. NAPI.—It is well known that this species is seasonally dimorphic, the most so of the three British species of Pieris. Professor Weismann ('Studies in Heredity,' by Professor Meldola) has recorded some experiments upon the summer brood, the result of which was that by exposing the pupa for a period of three months to a temperature of about 33° F. it was converted into the spring form, and, as might have been expected, my results are in general accordance with his, but with some difference of detail, more particularly in relation to the temperature during the latter part of the pupal stage, a point to which no particular importance seems formerly to have been attached.

Mr. Vine sent me, on the 20th May last, a pair taken near Hailsham. They paired once or twice afterwards, and eggs to the number of 32 were laid on a potted plant of *Cardamines*, on which the larvæ were fed. These hatched in about a week, and 31 pupated from the 19th to the 28th June. Four pupæ were placed at 90°, these emerged in 6 days; four more were exposed to the temperature of the room, averaging about 67°, and three of them emerged in from 12 to 13 days, one going over till next spring. There is no considerable difference between these two lots, which I shall therefore class together as 7 brought out at a high summer temperature. The remaining 23 were placed at 33° for from three to four months, and then divided, 12 being exposed to a temperature averaging 54°—about equal to the shade temperature of an

English May; of these all but 4, which have "gone over" to the spring, emerged in from about 36 to 40 days (with one exception, which was a few days more). The residue of 11 that had been iced, were placed at about 80°, and all but 3, which have "gone over," emerged in from 6 to 10 days. None were in any way crippled.

We thus have for comparison, individuals of the summer brood of three classes, *viz.*—(1) placed throughout at a high summer temperature; (2) first exposed to a severe artificial winter of 3 or 4 months, and then exposed to an artificial spring of 5 or 6 weeks; (3) first exposed to the artificial winter, and then to a high summer temperature for a week or ten days, without any intermediate spring. Class 1 was of course of the usual form of the summer emergence. A comparison of it with Class 2 shows that the effect of the artificial winter and spring on these pupæ, belonging naturally to the summer form, was to bring out most, but not all, of the characteristics of the spring form, thus—on the upper surface the greater suffusion, but less intensity of dusky colouring, especially along the nervures, and particularly in the females, and the characteristic colouring of the dark parts, *viz.*, grey instead of dark brown; on the under surface, faintness of the spots on the fore wings, the strong marking of the nervures of all the wings, and the stronger colouring of the yellow tinted parts.

In Class 3, *i.e.*, those exposed to a hot summer temperature immediately after a winter one, all these characteristics are noticeably less, especially the faintness of the spots on the under surface of the fore wings, but with one remarkable exception—that the nervures, especially on the hind wings, are more strongly marked than in either of the two other classes, the greater strength of the marking, however, being attributable more to the darkness of it than to an extension of its area.

The only general remark I will make on these results is that I think the experiments prove that some, but not all, of the characteristic colouring of this species depends, not on the particular emergence, *i.e.*, summer or spring, to which the insect when entering on the pupal stage belongs, but on the temperature to which the individual pupa is exposed. I have nearly 90 pupæ naturally belonging to the spring emergence on which I shall experiment during the approaching winter and spring, and may then perhaps have some more definite conclusions to bring forward.

It will have been observed that no less than 8 out of 31 of those *P. napi* which entered the pupal stage (*i.e.*, more than a fourth) had made their election, if I may use the expression, to belong to the spring emergence. This spontaneous splitting of a brood into the two emergences is a common occurrence (attributable, as I believe, generally to individual congenital causes), with many double brooded species, and notably with *P. napi*. Professor Weismann records ('Studies in Heredity,' by Meldola, pp. 39, 40) that a whole brood of his went over in this manner, and thinks this may have been owing to the mechanical motion to which they were subjected in a seven-hour railway journey, but I should question whether the occurrence was not rather to be ascribed to a congenital tendency happening in his case to affect all the individuals of the brood; in other species I have often known a whole brood go over in this way. His statement that the butterflies from eggs laid by the spring emergence of *P. napi* under ordinary circumstances always emerge in the summer, generally in July, of the same year, is I think not in accordance with the experience of many English collectors. Mr. Hawes informs me that of a large brood he had, more than half went over to the spring.

V. ATALANTA.—This is an insect which generally varies but slightly in appearance, and from which I should not have expected such results as have been in fact obtained.

I collected more than 100 larvæ with a few pupæ between 24th August and 16th October last. About 15 per cent. died from having been attacked by parasites.

My first temperature divisions were as follows:—

(1) At between 80° and 90°; these all emerged in 6 days.

(2) At the room temperature, 62° to 69°, averaging about 64°; these emerged in 18 to 19 days.

(3) In a cellar at an equable temperature of about 56°; emerging in about 34 days.

(4) In a cool place out of doors, at about 51° to 64°, averaging 54° or less; these emerged in 44 days.

(5) In the refrigerator, averaging about 45° by day, and in the room by night, averaging about 58°; 51° being about the general average, and the insects emerging in about 40 to 50 days.

Classes 2, 3, and 4, *i.e.*, those at temperatures from about 54° to

64° corresponding with such temperatures as may be met with in English summers, do not greatly differ *inter se*, but Class 1 at 80° to 90° differs appreciably from them.

Class 1, *compared with Classes* 2, 3, *and* 4. On the *upper surface* the black is not really black, but slightly suffused with golden brown, so as to give it a rusty look, and the scarlet bands are broad. At the three lower temperatures the black is intense, the scarlet band narrower, with a greater tendency to be broken up into parts on the fore wings and to be invaded by black scales at the anal angle in the hind wings; all the colours seem more intense, the intensity, I think, slightly increasing as between the 3 classes with the lowering of the temperature, and there is certainly with this lowering an increased development of the lavender-gray submarginal broken band. *On the under surface* of the *fore wings* of 2 out of the 12 at 90° a new small scarlet spot appears between the scarlet band and the inner edge. On the under surface of the *hind wings* of those at the three lower temperatures there is an even stronger intensification of colouring than on the upper surface, and especially of the dark colouring. The ground colour of the hind wings is decidedly much darker than in those at the higher temperature. On the other hand, the light parts are decidedly lighter and more strongly marked in those at the lower temperature. The chief of these light parts are—(1) a conspicuous cream coloured spot, something like a figure of 8 with the upper loop much the larger; this spot becomes more dense and conspicuous, and the loops have a tendency to be filled up and to spread along the costa, so as to give the spot a triangular form. (2) a light ochreous cloud near the middle of the hind margin. This becomes lighter and more strongly marked, and light cloudy markings begin also to appear near the centre of the hind wings. These effects on the under surface of the hind wings mostly increase with the progressive lowering of the temperature.

In Class 5 there is no great difference on the upper surface, the scarlet being, however, a little deeper in hue, but on the under side of the hind wings the effects of the low temperature are visibly enhanced—the creamy spot becoming denser and increasing in its tendency to spread along the costa, and the light cloudings becoming more marked and increasing in area, and even appearing in additional places. A few of these individuals show slight indications of suffering from the temperature.

Class 6. A sixth lot was kept wholly in the refrigerator at about 45° (corresponding with the latter part of an English November) for from 32 to 47 days, and then brought into various temperatures ranging from 90°, at which they emerged in 6 days more to

the temperature of the room, ranging from 48° to 63°, averaging about 55°, at which they emerged in from 34 to 19 days more, making in all from 40 to 70 or more days. Here the invasion of black on the *upper surface* has made greater progress, the scarlet band on the fore wings being narrowed and invariably broken up into several parts, and approaching carmine in colour, and there is a tendency, which in 3 specimens out of 7 is very strongly marked, for the white and lavender scales to spread so as to diffuse the edges of the white spots and to dust the black parts in patches with lighter scales. The blue at the anal angle of the hind wings, which is usually confined to one or two spots, in some of this class extends, but in a minute degree, and as lavender rather than blue, to nearly all the small black spots on the marginal scarlet band, and in two, but mostly so in the one figured (Fig. 5), there is on the extreme margin a row of minute blue or lilac spots alternating with minute marginal black spots on the fringe. On the *under surface* the tendency to spread is stronger, the metallic blue inverted U near the costa of the fore wings is scattered into a shapeless form, and the conspicuous "figure of 8" spot on the costa of the hind wings becomes an elongaged whitish cloud, in two specimens extending along the whole of the costal margin; the other light parts of the hind wings are lighter and more spread. The light colouring of the hind margin forms a broad ochreous marginal band, and is in several cases divided by a submarginal, well-defined darker line parallel with the outer edge, this line being in one case of a deep or tawny orange hue; and the whole of the central area of the hind wings has a blurred or clouded effect, produced by a suffusion of lilac scales dusted over the surface.

Those exposed to this low temperature (45°) for less than 45 days, though their colouring is much affected by it, do not seem to have suffered in vigour, but most of those exposed to it for longer periods died or were crippled.

Next, some experiments were tried by icing the pupæ, *i.e.*, placing them at a steady temperature of 33°. The result was uncertain. Little or no effect seems to have been produced on their colouring or markings by thus icing them for 47 days or less, and then exposing them to an ordinary summer temperature, though in many cases such icing caused death or crippling. I have one specimen iced 47 days, and then exposed to a temperature of 50° to 60°, when it emerged in 23 days more, making in all 10 weeks, and it is not to be distinguished from normal specimens. Of two iced 46 days, and then placed at an average temperature of 54°, and emerging in 29 days more, making in all nearly 11 weeks, one has

the scarlet band on the upper wings clouded with a whitish yellow colour, and that this is the result of temperature is shown by the other of the two, which exhibits a trace of the same colouring. There is also a tendency to yellowness of the scarlet band on the hind wings in some of those which are more or less crippled.

The results with *V. atalanta* may be summed up as follows :—

I. At 90°, pupal period 6 days (Fig. 3). *Upper surface.*—The black is rusty, the scarlet is of wide area and bright. *Under surface.*—The ground colour of the hind wings is brown, not dark brown, the light parts not very light. In two a new scarlet spot appears between the scarlet band on the fore wings and the inner edge.

II. At 64° *to* 51°, period 18 to 50 days (Fig. 4). *Upper surface.*—The black is intense, the scarlet rather deeper in colour and narrowed. *Under surface.* Ground colour of hind wings dark brown, light parts lighter.

III. At 45°, period 47 to 32 days, and then at various temperatures from 90° to an average of 55°, emerging in from 19 to 34 days more (Figs. 5 and 5*a*.) *Upper surface.*—Further invasion of scarlet by black, scarlet band further narrowed and broken up, and of a hue approaching carmine; spread of white and lavender scales over black parts, edges of spots diffused; minute blue or lilac spots on orange marginal band of hind wings increased in number. *Under surface.*—Great increase in lightness and area of light parts; diffusion of edges of spots and of some markings, and appearance of some new markings.

The results obtained by extreme and protracted cold *i.e.*, 45°), though probably such as would rarely be met with in nature, seem to me exceedingly interesting—first, as proving by this extreme case that the less marked intermediate results are caused by temperature; and secondly, because, owing to the great change in marking and colouring which they exhibit, especially on the under side of the hind wings, they may, it seems to me, possibly throw some light on the evolution of the markings in the Vanessas. This difference of appearance in these extreme cases is so great that I think if some of the insects could be seen in the resting position, *i.e.*, showing only the under side of the hind wings and the

comparatively uncoloured tips of the fore wings, they would be taken as belonging to different species.

I may here make a general observation on the minute white spot on the scarlet band which has been the subject of some discussion. About one in four of mine shows this spot or traces of it on the upper surface; the whole of them—over 60 in number—show it or faint traces of it on the under surface. Its existence does not seem dependent on temperature.

Hibernating stage of atalanta.—This species is well known to hibernate in the perfect condition, coming out in May or June, and then pairing and laying its eggs on nettle, the larvæ being found through July and August, and the fresh butterflies from them appearing through the latter part of August and September, and being seen as late as October or even November, and then going into winter quarters. Mr. Scudder states that in the south of Europe and in the southern part of the United States it has two or three broods, and that even in the northern States some pupæ of a second brood hibernate, and the butterflies emerging from them mingle with the butterflies which have hibernated. My experiments rather confirm the ordinary opinion that in England none hibernate as pupæ, though I have no doubt many of the butterflies of the summer emergence pair and lay eggs, resulting in butterflies which emerge in the late summer or autumn and hibernate, for I have found young larvæ as late as 16th October, and I have now a pupa from one which did not pupate till 16th November. But of all the pupæ which I subjected to a low temperature none have yet survived more than about 11 weeks. My last perfect specimens emerged 26th and 27th November.*

C. PHLÆAS.—I have been for some time desirous of experimenting on this species, because it is known that, while not considered materially affected by the great difference in temperature between a summer and a winter in Central Europe, it is apparently affected by the somewhat higher temperature of Southern Europe, the summer emergence having there a tendency to be suf-

* Note of 31st December. I subjected about thirty to winter conditions, some placed out of doors shortly after pupation; all these have died.

fused with dusky scales; an example of this is figured in Weismann's work before quoted.

By the kindness of friends, Mr. Fletcher and Mr. Nicholson, I was last spring supplied with some living specimens of the butterfly, which laid eggs on dock and sorrel, and from them I had more than 70 pupæ, which proved exceedingly healthy. Ten of these were forced at 85° and 10 at 80°, all emerging in from 6 to 7½ days. There is little difference between these two lots, which are remarkable for the large size of the spots, and the comparative dulness of the colouring, the black parts being less intense, and the coppery parts especially having a slightly brassy appearance, and being in most cases more or less suffused with dusky scales, especially towards the bases of the fore wings, and often on the nervures; the coppery band on the hind wings is also narrow and broadly serrated. Six were brought out at the temperature of the room, averaging about 70°,—fairly representing an English summer temperature,—emerging in 11 to 15 days. These are noticeably different—the colours are more intense, the dark parts blacker, the coppery parts more vivid, and the spots smaller. Another lot of 6 was placed in a cool and shady place out of doors, the temperature averaging about 58°, *i.e.*, about the mean shade temperature of the latter part of May, and emerged in from 22 to 30 days; these show a slight further increase in the brightness of the coppery parts, especially towards the bases of the fore wings, the spots are, I think, still smaller, and the coppery band on the hind wings is broader. Another lot, of 10, was placed in a cellar at a uniform temperature of about 56°, emerging in from 29 to 33 days; these are very similar to those last mentioned. Six more were placed in the refrigerator at about 47°, representing the shade temperature of the earlier part of an English April, from which they evidently suffered, for only 3 emerged, in from 57 to 59 days, and one of these is unsymmetrical, one wing having a rather silvery hue; these 3 show a still further advance in the lightness (but not in the brightness) of the coppery parts, and certainly in the breadth of the coppery band on the hinder wing.

More were kept at a temperature of 33° for about 10 weeks, and then brought into a temperature averaging about 55°, thus representing winter conditions followed by spring, and emerging in from 34 to 36 days more—*i.e.*, in all about 15 weeks. About half died or were crippled. In those which emerged all the effects of the low temperature are seen in their extreme—the light colour of the coppery parts, the reduced size of the spots, one or two of

which have almost disappeared, and the breadth and conspicuousness of the coppery band on the hind wings, which ceases to be serrated, though the coppery scales are often prolonged along the nervures from the band towards the base of the wings. I tried a further experiment with some iced at 33° for 9 or 10 weeks, which I consider sufficiently proves that the dusky suffusion is, at least to some extent, the effect of a moderately low temperature extending over some of the later pupal stages. Six of those iced for about 10 weeks were transferred at once to a temperature of 90° ; these all emerged in from 5 to 6 days, and it will be seen that they have most of the features of those that were never at a lower temperature than 80° to 90°, and especially the suffusion of the bases of the fore wings with duskiness, and the reduced size of the coppery band on the hind wings. The contrast in colouring between the last two lots, the only difference between them in treatment being that, both having been long iced, one lot emerged at 55° while the other emerged at 90°, is noticeable.

The only individuals which seemed at all injured by the temperature applied were those at 47°, and those which after long icing were placed at about 55°. Mere icing for 10 weeks did not seem at all injurious.

I will summarise the results as follows :—

I. At 80°—90°. Representing a very hot continental summer temperature (Figs. 1 and 1*a*). Emerged in 6 days. Spots large, not sharply defined ; dusky suffusion of fore wings.

II. At about 70° (English summer temperature). Emerged in 11 to 15 days. Spots smaller, copper colour more vivid, black more intense.

III. At about 56° *to* 58° (rather cool summer or late spring temperature). Emerged in 22 to 23 days. Copper colour still more vivid ; copper band on hind wings broader. .

IV. At about 45° (temperature of cold spring). Emerged in 57 to 59 days. Effects intensified.

V. At 33° *for* 10 *weeks, then at* 55° *for* 5 *weeks* (winter and spring temperatures) (Figs. 2 and 2*a*). Extreme effects produced, especially in smallness of spots and breadth of the coppery band on hind wings.

VI. At 33°, *then* 90° for 6 days (winter, immediately followed by very hot summer). Re-appearance of dusky suffusion and of narrowness of copper band on hind wings.

It seems to follow that in this species the principal effects on colour, &c., are produced not by long exposure to severe cold, but by exposure, during the period when the active part of the pupal stages has begun, to (1) great heat, producing duskiness, or (2) moderate cold, producing vividness and intensity of colouring in both the coppery and the dark parts, smallness of spots, and great enlargement of the copper band on the hind wings.

These experiments show, I think, that the difference in appearance between *phlœas* from Southern Europe and *phlœas* from England is not necessarily to be attributed to the existence of races of different colouring, but may be owing to the difference between the temperatures to which the individuals are exposed in the two climates.

My results show an interesting parallelism with those produced, apparently by corresponding natural differences of temperature, in the American copper, *hypophlœas*, which nearly resembles *phlœas* in appearance. In this species Mr. Scudder remarks that the spring individuals [*i.e.*, those which emerge in the colder weather] are of a more fiery red, and the orange band on the under surface of the hind wings is broader; while in later broods [*i.e.*, those emerging in the hot American summer] the markings are less vivid and less distinctly marked. He adds that there is a longer tooth on the margin of the hind wings; this feature appears to exist in a slight degree in my *phlœas* brought out at a high temperature; the orange band on the under surface of the hind wings is in all mine very inconspicuous. I am indebted to Mr. Frohawk for the observation that the markings on the under sides of all the wings are considerably stronger in those exposed to the high temperature than in those at lower temperatures, and in those at the lowest temperature the spots on the fore wings are much reduced in size, and on the hind wings are almost obliterated.

Ephyra punctaria.—This species is well known for its seasonal dimorphism.

Mr. Fletcher sent me a female taken in the New Forest, which reached me 4th June last, with from 140 to 150 of its eggs, which were just hatching, and from which I have bred 113 moths, besides 7 pupæ which have gone over till next spring. 21 of these

moths were from pupæ at about 90°, emerging in from 4 to 5 days; 22 at the temperature of the room, about 70°, emerging in from 10 to 11 days; 17 in a cellar at a regular temperature of about 56°, emerging in 22 to 27 days; 13 in the refrigerator at a temperature averaging 45°, and emerging in from 57 to 70 days. In all the lots there was a preponderance, generally a large one, of females.

There are not many differences in appearance between those at 90° and those at 70°, the chief ones being that those at the lower temperature are rather more sprinkled with dark scales, giving them the appearance of a slightly darker ground colour, and that all the markings are also slightly darker; both generally show the "inner" line as well as the "central" and "outer" lines; and, in both, nearly every specimen, male and female, shows very conspicuously the blotches usual with the summer emergence between the outer line and the margin; in those at 90° all but one are thus blotched, in those at 70° all but about 4. Coming to those brought out at 56°, there is a slight increase in the dark ground colour, the central line has become conspicuously darker, and the disappearance of the blotches has made further progress, only 7 or 8 individuals out of 17 showing them. Passing on to those at 45°, the ground colour is again slightly darker, and the central line darker still, while the blotches have disappeared, leaving indeed very faint traces in about 3 out of 13.

A large number were kept in ice at 33° for three months, and were then placed out of doors (being in cold weather brought indoors), the temperature averaging about 54°, and the moths emerging in from 36 to 42 days more; these are very like those brought out at 56° without icing; only 4 out of 20 are blotched, and these but faintly.

Of those iced at 33° for 3 months, 19, placed at 87°, emerged in from 5 to 7 days. These are remarkable for a near return in appearance to those which were never at a lower temperature than about 90°—in general lightness of colour, owing to the sparseness of dark sprinkling, and in the reappearance of the blotches, 14 of the 19 being blotched, some conspicuously so.

I may add that on the under sides most of these effects may be faintly traced.

None seem to have suffered in any way from the temperature except those at 45°, nearly a quarter of which died or were crippled.

To sum up. We have in *E. punctaria* a gradual disappearance of the submarginal blotches, increase of dark sprinkling, and intensification of the central line, as the temperature is lowered from 90° through 70°,

and 56° to 45°. A temperature of 33° seems to suspend the physiological changes without much, if any, other effect, for those which were thus iced for over 3 months and then exposed for from 5 to 7 days to a high temperature, closely resemble in appearance those exposed to a similar temperature without having been iced at all.

Explanation of Plate IV.

Figs. 1 and 1*a*. *C. phlæas*, at 80°—90°, emerging in 6 days.

2 and 2*a*. *C. phlæas*, at 33° for 10 weeks, then at 55°, emerging in 5 weeks more.

3. *V. atalanta*, both sides, at 90°, emerging in 6 days.

4. *V. atalanta*, under side only, at about 45° to 58°, emerging in 40 to 50 days.

5 and 5*a*. *V. atalanta*, at 45° for 42 days, then at about 48° to 63°, emerging in 19 days more.